Journal of Medical Virology 55:255–261 (1998)

Immunization of Common Marmosets With Epstein-Barr Virus (EBV) Envelope Glycoprotein gp340: Effect on Viral Shedding Following EBV Challenge

Charles Cox,[1,2] Beverley A. Naylor,[1] Mike Mackett,[3] John R. Arrand,[3] Beverly E. Griffin,[2] and Nina Wedderburn[1,2*]

[1]*Department of Pathology, the Royal College of Surgeons of England, London, England*
[2]*Department of Infectious Diseases, Division of Investigative Science, I.C.S.M. Hammersmith Hospital, London, England*
[3]*Department of Molecular Biology, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Withington, Manchester, England*

Epstein-Barr virus (EBV), the cause of infectious mononucleosis, is involved in the pathogenesis of several human cancers, the highest frequency of association being found in undifferentiated nasopharyngeal carcinoma and endemic Burkitt's lymphoma. The development of animal models in which potential vaccines can be tested is important. EBV infection of the common marmoset, using the M81 strain originally derived from a patient with nasopharyngeal carcinoma, induces a carrier state in this animal. Persistent infection is characterized by the production of antibodies to viral antigens, and the secretion of EBV DNA into buccal fluids. Following immunization with envelope glycoprotein gp340 derived from a bovine papilloma virus expression vector, prior to EBV infection, viral DNA was detected significantly less frequently in the buccal fluids of immunized, than of nonimmunized, infected animals, indicating that although the carrier state had not been abolished, it had been altered. A reduction in virus load was also observed when offspring of seronegative, and on occasion seropositive, parents were immunized neonatally, before EBV challenge. ***J. Med. Virol. 55:255–261, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: EBV vaccine; PCR; buccal fluids

INTRODUCTION

Work on potential vaccines to reduce the incidence of human disease associated with the Epstein-Barr virus (EBV) has focused on the major envelope glycoprotein, gp340, antibodies to which are virus-neutralizing [Pearson et al., 1970; Epstein, 1976]. When combined with one of several adjuvants, or expressed in recombinant virus vectors, gp340 protects against the lethal effects of EBV challenge in the cotton-top tamarin, *Saguinus oedipus* [Cleary et al., 1985; Morgan et al., 1989; Morgan 1992; Ragot et al., 1993]. The degree of protection afforded by different preparations of gp340 varies and, interestingly, is not correlated with virus-neutralizing antibody [Epstein et al., 1986; Morgan et al., 1988]. Recombinant gp340/220 from a bovine papillomavirus (BPV) expression vector [Madej et al., 1992], combined either with a threonyl muramyl dipeptide [Finerty et al., 1992], or alum [Finerty et al., 1994], provides protection of some, but not all, animals. Although the protected tamarins survive what is usually a lethal dose of virus, a sterilizing immunity is not induced, indicating that persistent EBV infection is not prevented by prior immunization [Niedobitek et al., 1994].

EBV does not produce clinical disease in the common marmoset, *Callithrix jacchus,* although by using the M81 virus strain [Desgranges et al., 1976], long-term production of antibody to viral antigens was shown to follow experimental infection [Wedderburn et al., 1984]. M81 is a type A virus originally derived from a patient with nasopharyngeal carcinoma; it lacks the deletion seen in the *Bam*HI I region of the genome in the B95-8 strain [Parker et al., 1990; Mackett et al., 1996]. In two animals infected experimentally as neonates, IgG antibody to both virus capsid (VCA) and early antigens (EA) gradually increased, and was fol-

Contract grant sponsor: the Cancer Research Campaigne; Contract grant sponsor: the Royal College of Surgeons of England.

*Correspondence to: Nina Wedderburn, Department of Infectious Diseases, Division of Investigative Science, I.C.S.M. Hammersmith Hospital, London W12 ONN, UK.

Accepted 23 February 1998

© 1998 WILEY-LISS, INC.

lowed by production of IgA antibodies persisting over a period of nine years. Whole-mouth fluid (WMF) positivity was found at a high level in every sample tested from these animals [Cox et al., 1996]. Naive adult animals can be infected naturally by their cagemates, as indicated both by seroconversion and by the production of EBV DNA in (WMF) [Cox et al., 1996]. Infection of babies by their parents, and of young weanlings placed in a commune with a seropositive animal (unpublished), also occurs. In the majority of both experimentally and naturally infected adult marmosets, shedding into buccal fluids was found to be intermittent over a period of at least two years [Cox et al., 1996; Cox, 1997]. Recently small nuclear EBV-encoded RNAs (EBERs) have been demonstrated in peripheral lymphocytes of these animals [Farrell et al., 1997]. Taken together, these results indicate that, as in man, a carrier state can be established in the common marmoset. We have thus explored the value of this animal as a model for studying vaccines.

We describe the effects of glycoprotein 340/220 [Madej et al., 1992] together with alum, given prior to virus infection, on the subsequent production of antibody, and the incidence of viral shedding into buccal fluids. The immunizing gp340 used was generated from B95-8 DNA, but the 5′ 181 amino acids of the molecule where the major conformationally sensitive, virus-neutralizing epitope is mapped [Tanner et al. 1988] are virtually identical between M81 and B95-8 strains, containing only a single conservative amino acid change [Mackett et al., 1996]. Both immunized adult animals and neonatal marmosets, offspring of seropositive and seronegative parents, respectively (immunized before weaning), were challenged subsequently with EBV, and the course of infection followed.

MATERIALS AND METHODS

Animals

The common marmosets used in this study, *Callithrix jacchus,* were part of a closed colony to which there had been no additions since 1970.

Vaccine

Recombinant gp340/220 was derived from a bovine papillomavirus (BPV) expression vector [Conway et al., 1989; Madej et al., 1992]. Three parts of gp340 at 67 μg/ml were mixed with one of Alhydrogel (alum) {v/v} [Superfos Denmark, Grade A, 1.3% $Al(OH)_3$], left overnight at 4°C, spun down, and the pellet resuspended in saline (sodium chloride injection, BP, 0.9% w/v, Evans Medical Ltd, UK). Three doses were given intramuscularly (i.m.) at 4-weekly intervals. Adults received 30-μg and neonatal animals 5-μg gp340 per dose.

Epstein-Barr Virus

EBV was obtained from the M81 cell line[Desgranges et al., 1976]. Supernatants were concentrated by centrifugation [Johnston et al., 1990]. Animals were infected with EBV orally by injection into the palatine tonsils, the back of the tongue, and sublingually, unless otherwise stated, under ketamine anesthesia (10 mg/kg i.m., Vetalar, Parke Davis, Gwent, UK). Inactivated EBV was prepared by incubating at 37°C in 1: 2,000 formalin for 72 hr, with occasional shaking, neutralizing with $NaHSO_3$, and dialyzing against dH_2O at 4°C for 24 hr.

Blood and Buccal Fluids

Blood for hematological investigations was taken under ketamine anesthesia. Whole-mouth fluid (WMF) was obtained by inserting 0.3-ml dH_2O into the mouth under ketamine anesthesia and removing the fluids.

Hematological Investigation and EBV Serology

Differential white-cell counts were carried out on May-Grünewald-Giemsa-stained blood smears. Heterophile antibodies were detected using a rapid slide test (Mercia Brocades, Surrey, UK). Antibodies to virus capsid antigens (VCA) were estimated by indirect immunofluorescence on P3HR1 cells [Henle and Henle, 1966]. Although this test is predominantly used for the determination of VCA antibodies, in immunized animals it also registers a different pattern of antibody response to gp340 (see Fig. 1B), and is therefore designated as a response to virus lytic antigens (VLA). Antibody to gp340 was assayed by ELISA, as described by Randle and Epstein [1984], with minor modifications; 0.1 μg of gp340/220 (derived as above) was adsorbed per well. Results are expressed as the mean optical density (OD) (490 nm) of a 1:10 dilution of serum after subtraction of background.

PCR

Primers used in polymerase chain reaction (PCR), LLW1 and LLW2 [Labreque et al., 1995], localized between base pair (bp) 505 and 740 [Baer et al., 1984] in the repetitive *Bam*HI W EBV fragment, yield a product of 236 bp. LLW1 is made up of 5′-CCATGTAAGCTTGCCTCGAG-3′; LLW2, 5′-GCCTTAGATCTGGCTCTTTG-3′. Primers Del.1 and Del.2 [Lees et al., 1992], from a unique sequence of the viral genome, were used in some instances for confirmatory work.

PCR protocol. WMF (25 μl) was diluted with 75 μl of dH_2O, boiled for 7 min, and chilled on ice. Twenty μl were then added to a reaction mix containing 2.5 units Taq polymerase (Perkin-Elmer-Cetus, Cheshire, UK), 20-mM $(NH_4)_2SO_4$, 75-mM Tris-HCl (pH 9.0), 1.5-mM $MgCl_2$, 0.01% (w/v) Tween 20, 100 pM of each primer, and 25-mM dNTPs. PCR was carried out using 35 cycles, comprising denaturation at 95°C for 1 min, and combined annealing and extension at 63°C for 3 min. A final step was undertaken at 63°C for 5 min. Products were electrophoresed on a 2% agarose gel, transferred by Southern blotting to a nylon membrane (Biodyne B, Pall) after NaOH denaturation, and the membrane was baked at 80°C for 1 hr. Hybridization was carried out using an [α^{32}P] dCTP radiolabeled DNA probe specific for the *Bam*HI W fragment of the EBV genome, in a Bluescript vector (Stratagene, La Jolla, CA). DNA was labeled with the Random Primers

TABLE I. Immunization of Adult Marmosets Prior to EBV Challenge

	Animal	Immunizations (–12, –8, and –4 weeks)	EBV challenge (0 and 12 weeks)
M	827, 859	Alum only	3×10^4 ID_{50} (oral)
F	820, 822, 832, 836		
M	857, 867	30-μg gp340/alum	3×10^4 ID_{50} (oral)
F	817, 821, 829		

DNA Labeling System (Gibco BRL, Scotland, UK), using 50 μCi [α^{32}P] dCTP (2–3 × 10^3 Ci/mM, Amersham, Life Sciences, UK) for each probe. Hybridization was carried out overnight in 5 × Denhardt's solution, 6 × SSC, 0.1% w/v SDS, 1.5-mM sodium pyrophosphate, 25-mM NaH_2PO_4, and 100 μg/ml of sonicated calf thymus DNA. Three one-hour washes with 0.3 × SSC, 0.1% SDS were followed by two 30-min washes with 0.1 × SSC, 0.1% SDS. The membrane was left in contact with X-ray film (Fuji, Japan) for one hour to one week at –70°C before developing [Cox et al., 1996].

Removal of PCR inhibitors. PCR inhibitors were assessed by comparison of WMF samples spiked with varying numbers of M81 cells (either 10^3, 10, or 0 cells) with $d$$H_2O$ controls spiked with identical numbers of these cells. If the signal produced by 10 M81 cells in an individual WMF sample was less than that produced by 10 M81 cells in $d$$H_2O$, then the sample was treated with Chelex 100 (Biorad, Herts, UK). Five percent (w/v) Chelex 100 25 μl in $d$$H_2O$ (or for strongly inhibitory samples 25–200 μl 25% (w/v)) was added to 25-μl WMF and 75-μl $d$$H_2O$, and the mixture incubated at 56°C for 30 min, vortexed for 10 sec, boiled for 5 min, and microfuged for 4 min at room temperature. Once all traces of inhibitors had been removed, a semiquantitative estimate of the amount of EBV DNA present in a given WMF sample could be made by comparing the signal detected in the unspiked WMF sample with that produced from 10 M81 cells in $d$$H_2O$. Signals less intense than, roughly equal to, or stronger than that found with this standard were assigned values of +, ++, or +++, respectively, as described previously [Cox et al., 1996]. Some samples assigned a value of +++ produced a stronger signal than that found with 10^2 M81 cells, while a few, as assessed by slot-blot hybridization, appeared to contain EBV DNA equivalent to 10^3–10^4 cells; duplicates, where tested, showed a high degree of reproducibility [Cox et al., 1996; Cox, 1997]. When samples were retested using primers specific for the *Bam*HI Ib region of the Raji genome (Del.1 and Del.2 [Lees et al., 1992]), values assigned to experimental samples relative to the 10 M81 cell standard did not differ significantly from those obtained using primers for *Bam*HI W.

RESULTS

Preliminary experiments were carried out to establish a reproducible method of oral EBV infection in common marmosets. A single dose of 3×10^4 ID_{50} of M81 EBV, swallowed without breaching the oral mucosa, was followed by seroconversion, but VCA titers usually appeared only months later, and remained low, resembling the response seen in natural cage infection [Cox et al., 1996]. The same dose, injected into the tongue, the palatine tonsils, and sublingually, gave rise to moderate to high titers of antibody to VCA, which persisted for at least a year. Injection of a lower dose (3×10^3 ID_{50}) also caused VCA positivity, but titers remained very low (≤10) for at least a year while injection of 3×10^2 ID_{50} EBV was not followed by seroconversion.

In the first experiment described below, two oral injections of 3×10^4 ID_{50} EBV were given with a 12-week interval; in the subsequent experiment a single dose was found to give rise to persistent infection. When formaldehyde-treated virus was used, comparable titers of VCA developed, but only persisted for 4–5 months. The early response to infection by the oral route differed from that observed following intraperitoneal (i.p.) and intramuscular (i.m.) infection [Wedderburn et al., 1984]. In the majority of animals, oral infection did not give rise to a positive Paul Bunnell reaction, and although sporadic lymphocytosis was observed, this did not present as a predictable, significant rise in the circulating lymphocyte count, 6–8 weeks after infection.

Immunization and EBV Challenge of Marmosets

Five adult marmosets were given gp340 with alum, and six others alum only, before challenge with EBV (Table I). Most animals were housed, for at least part of the experiment, with a partner from the same experimental group; within any group pairings were sometimes changed. One marmoset (820) (see Table II) was caged alone, after rejecting various pairings.

Sequential samples of WMF were analyzed by PCR. All samples were negative for EBV DNA before virus infection; all were positive after four weeks. At eight weeks the two groups diverged and at twelve weeks all immunized animals scored negative. After the second EBV challenge, this degree of protection was not maintained and all animals produced positive samples intermittently for the next 36 weeks. In these small samples, no significant differences were observed between male and female animals. The data are summarized in Table II. The numbers of positive samples were compared, but without reference to the degree of positivity. A consistently lower incidence of EBV DNA in WMF was observed in immunized (47%) than in nonimmunized (74%) animals ($P \leq 0.001$, χ^2 test). When individual animals were ranked by the number of posi-

TABLE II. PCR Analysis of Sequential WMF Samples[a]

Weeks (post-EBV)	Nonimmunized animals[b]						Immunized animals[c]				
	820	822	827	832	836	859	817	821	829	857	867
–12	---	---	---	---	---	---	---	---	---	---	---
0	---	---	---	---	---	---	---	---	---	---	---
2	++	++	++	+	+++	+++	+++	++	+++	++	---
4	+	++	+	++	+++	+++	+++	++	++	+++	+
8	+++	---	++	---	+	+	---	+	++	---	---
12	++	---	++	---	---	+++	---	---	---	---	---
14	++	+++	+++	++	---	+	---	+++	+	+++	---
16	+++	+++	---	+	+++	+	++	---	---	+	---
20	---	++	+++	+++	---	++	---	---	---	+++	---
24	++	+	++	---	+	---	+++	---	---	+	---
28	++	++	+++	---	ND	+	---	---	++	---	+++
32	---	---	++	+++	+++	---	++	---	++	---	+++
37	++	---	+++	++	+++	---	+++	---	---	+	+
41	---	---	++	---	+	+++	---	---	+	---	+++
45	++	+	+	+	+	---	---	+	---	---	+
50	++	+	+	---	ND	+	---	+	---	---	+
Total	11/14	9/14	13/14	8/14	9/12	10/14	6/14	6/14	7/14	7/14	7/14

[a]WMF positivity was assessed as +, ++, and +++ (see Materials and Methods; also Cox et al. [1996]); --- indicates a negative sample; ND indicates not done.
[b]Total + ves = 61/82 (74%).
[c]Total + ves = 33/70 (47%).

tive samples each produced, the differences were also significant ($P \leq 0.0062$, Mann-Whitney U test).

Antibody to gp340 in these animals was assayed using an ELISA. The results are shown in Figure 1A. Immunization produced a strong anti-gp340 response that increased after infection; following the second EBV challenge, nonimmunized animals also responded. The antibody response of immunized animals, estimated on P3HR1 cells by immunofluorescence, contained more than one component. In addition to the usual deep-green cytoplasmic fluorescence, a low-titer membranous staining was observed, probably due to a response to gp220 and gp340, both of which are present in the vaccine and are expressed on the membrane of P3HR1 cells [Pearson and Luka, 1986]. Following EBV challenge, considerable increases in cytoplasmic fluorescence were observed (Fig. 1B). The titers are designated as antibody to viral lytic antigens (VLA).

Correlation was observed between the responses of individual animals to gp340 and to VLA. Although the WMF positivity of individual, nonimmunized animals varied, this was not related to antibody titers to either antigen.

At birth, offspring of EBV-seropositive mothers have anti-VCA titers that do not differ significantly from the maternal titer; these decline with time (Fig. 2). All six animals tested at twelve weeks had titers of <5. Some animals remained seronegative, while others seroconverted. IgM antibody was present briefly in some cases (data not shown).

In a second set of experiments, the effect of immunizing young offspring of either seronegative or seropositive parents was investigated. One of each pair of twin marmosets received gp340 and one received alum only. In contrast to the experiment with adults, young animals from different experimental groups were housed together. Immunization was carried out at 2, 6, and 10 weeks of age, before weaning. At 14 weeks they were separated from their parents and infected with a single dose of EBV (see Table III).

In spite of the fact that they received only a single EBV challenge, offspring of seronegative parents reacted similarly to adult animals. Twelve weeks after challenge no immunized animal had an EBV-positive WMF, and two of three unimmunized animals were also negative, but later, intermittent secretion recurred in both groups, indicating that this event did not depend on a second EBV challenge (Table IV). Forty-four percent of WMF samples from immunized, and 78% of samples from nonimmunized animals were positive for EBV DNA ($P \leq 0.04$, χ^2 test; $P \leq 0.05$, Mann-Whitney U test).

With offspring of seropositive parents, all three nonimmunized animals had anti-VCA titers of 5–20 before EBV challenge, and low titers of antibody to viral lytic antigens were present in the immunized group. The WMF of one animal from each group was EBV-positive by PCR before challenge (see Table IV). These results indicate that natural infection probably preceded EBV challenge. Offspring of seropositive parents will have been exposed to low levels of infectious virus during immunization, against a background of maternal antibody. Although immunization resulted in a reduction in the number of positive WMF samples from 67% to 53% following experimental challenge, this difference was not deemed significant. Antibodies to VLA did not differ significantly between the immunized and unimmunized groups.

DISCUSSION

Recent work supports the assumption that EBV persists mainly in B-cells [Gratama et al., 1988; Anagnostopoulos et al., 1995], specifically in resting cells [Miyashita et al., 1997]. While a very considerable varia-

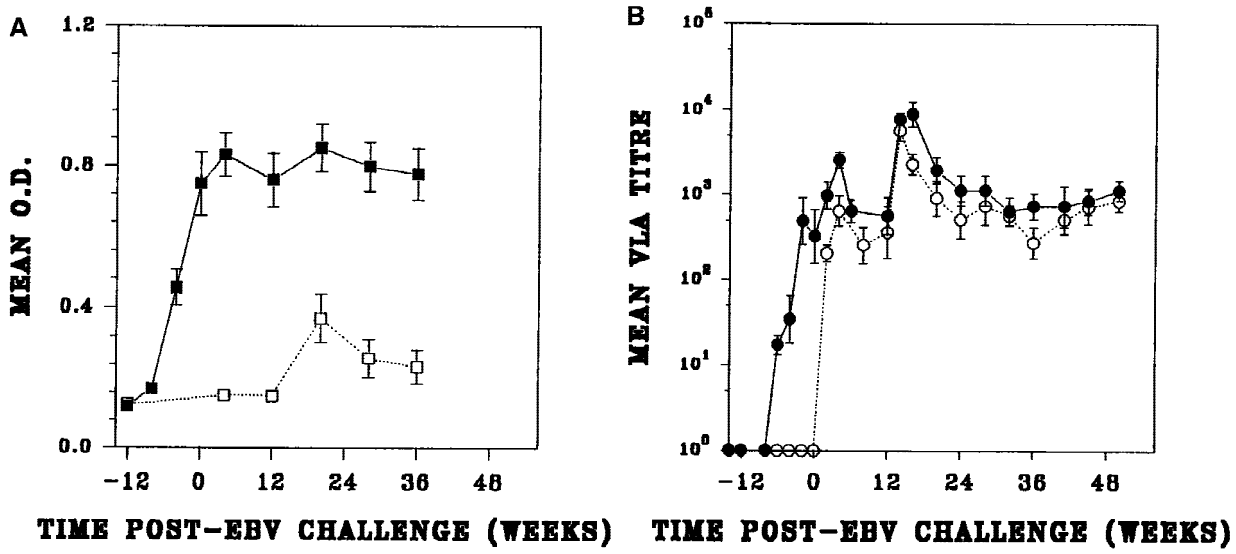

Fig. 1. **A:** Antibody to gp340 (mean OD [490 nm] ± standard error) in sera from six marmosets immunized with alum (□) and five with alum/gp340 (■), immunized and infected as in Table I. **B:** Mean anti-VLA titers (±standard error) in sera from six marmosets immunized with alum (○) and five with alum/gp340 (●), treated as in 1A.

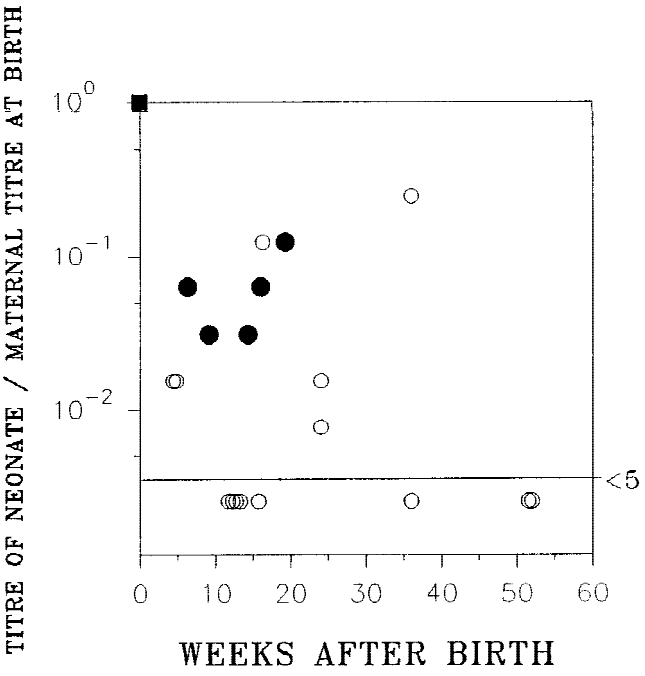

Fig. 2. Anti-VCA titers in offspring of seropositive parents, expressed as the ratio of neonate's titer/maternal titer at time of birth. One animal had five readings (●), eight had two (○), and one (taken at time 0) had one (■).

tion in the number of infected B-cells (×50-fold) is detected between healthy individuals, the frequency in any given individual hardly varies [Khan et al., 1996]. A direct relationship has been demonstrated between the levels of virus in circulating B-cells and EBV shedding in throat washings. This relationship is usually stable, but can be altered, for example by acyclovir treatment, which does not reduce the number of infected B-cells but temporarily abolishes viral shedding. When treatment ceases, shedding is reestablished [Yao et al., 1985a, 1989]. A similar relationship is observed in transplant recipients, although here viral loads are higher [Yao et al., 1985b].

In the marmoset model, the incidence of detectable EBV DNA in buccal fluids was chosen as a marker of EBV replication, and the effect of prior immunization on virus shedding was investigated. Immediately following oral EBV injection, high levels of viral DNA were observed in buccal fluids [Mackett et al., 1996] (Tables I and II), but these fell rapidly, indicating that this DNA might have come from the inoculum; similar high initial levels were not observed after natural infection [Cox et al., 1996]. In no case was the subsequent very low incidence of secretion observed 12 weeks after infection, maintained, regardless of whether a second EBV challenge was given at this point (Tables I and II, and unpublished results). Intermittent EBV DNA secretion was maintained for long periods, in two cases for nine years [Cox et al., 1996]. Immunization prior to EBV challenge, both in the independent experiments carried out here and in a previous study in which marmosets were treated with a recombinant vaccinia expressing gp340 [Mackett et al., 1996], caused a reduction in the incidence of secretion of EBV DNA, but not a sterilizing immunity.

In the common marmoset, the humoral response to EBV primarily depends on the dose and route of viral infection. Following initial VCA responses, titers remain fairly stable for 1–2 years, although in the longer term they may rise [Wedderburn et al., 1984; Cox et al., 1996; Mackett et al., 1996]. Similar very-long-term stability of anti-VCA titers was found in a prospective study of Burkitt's lymphoma [de Thé et al., 1978]. Emini et al. [1989], also working with marmosets, reported a lack of correlation between protection and the

TABLE III. Immunization of Young Marmosets Prior to EBV Challenge

	Animal	Status of parents	Immunization (−12, −8, and −4 weeks)	EBV challenge (week 0)
M	1045, 1049	Seronegative	Alum only	1.5×10^4 ID_{50} (oral)
F	1055			
M	1046, 1054	Seronegative	5-μg gp340/alum	1.5×10^4 ID_{50} (oral)
F	1050			
M	1051, 1056	Seropositive	Alum only	1.5×10^4 ID_{50} (oral)
F	1033			
M	1052, 1058	Seropositive	5-μg gp340/alum	1.5×10^4 ID_{50} (oral)
F	1034, 1053, 1058			

TABLE IV. PCR Analysis of Sequential WMF Samples From Young Offspring of Seronegative and Seropositive Parents[a]

	Seronegative parents						Seropositive parents							
	Non-immunized[b]			Immunized[c]			Nonimmunized[d]			Immunized[e]				
Week (post-EBV)	1045	1049	1055	1046	1050	1054	1033	1051	1056	1034	1052	1053	1057	1058
0	---	---	---	---	---	---	---	+	---	++	---	---	---	---
4	+++	++	++	++	---	+	+	+++	+++	+	++	---	+	+
9	++	+	+	---	+	---	---	++	++	---	---	+	---	+++
12	---	++	---	---	---	---	+	---	+	---	---	++	---	---
16	+	---	++	---	+	+	+	++	+	+	++	---	+	---
20	+	+	+	---	+	---	---	+	---	+	++	---	---	++
24	+++	++	---	+	---	+	---	---	+	---	+	---	++	---
Total	5/6	5/6	4/6	2/6	3/6	3/6	3/6	4/6	5/6	3/6	4/6	3/6	3/6	3/6

[a]WMF positivity was assessed as +, ++, and +++ (see Materials and Methods; also Cox et al., 1996); --- indicates a negative sample. Total indicates a number of times a given animal had EBV DNA present in WMF after EBV challenge.
[b]14/18 (78%).
[c]8/18 (44%).
[d]12/18 (67%).
[e]16/30 (53%).

development of neutralizing antibody, where protection against very high doses of B95-8-derived EBV was defined as complete abolition of the VCA response in animals previously treated with gp340 and alum. We could not confirm their findings. Indeed, a complete lack of response to the B95-8 virus used is difficult to account for, since even the injection of formalized virus was found to result in the development of antibody to VCA [Emini et al., 1986]. On the other hand, treatment with a recombinant vaccinia expressing gp340 was found to prevent or delay the emergence of antibodies to VCA in children who were exposed to a natural EBV infection, probably at a lower dose and by a different route from that used experimentally [Gu et al., 1995; H. Wolf, personal communication].

Classical vaccines may prevent clinical disease without preventing initial infection and viral replication. It is in the light of this that attempts to vaccinate against agents such as EBV, which become latent or establish persistence, should be viewed [Spring et al., 1996]. Successful vaccination against experimental lentivirus infection of primates has been reported in terms of delayed disease [Sutjipto et al., 1990], or by a gradient of suppression of growth of the challenge virus from sterilizing immunity to a decrease in virus or provirus burden [Israel et al., 1994]. In the context of primary infection with EBV, a decrease in virus replication following immunization may well prevent infectious mononucleosis in adolescence, and act to decrease the severity of disease in the EBV-negative recipients of a positive transplant. The primary infection in very young African children, which results in exceptionally high VCA titers [de Thé, 1982] might also be modified, leading to a lowering of the number of children at risk of developing Burkitt's lymphoma. In large part, our work assumes a need for an EBV vaccine and has focused on elaborating a useful model for testing it prior to delivery to man.

ACKNOWLEDGMENTS

We thank Dr. David Wood and Dr. C. Doré for helpful advice.

REFERENCES

Anagnostopolous I, Hummel M, Kreschel C, Stein H (1995). Morphology, immunophenotype, and distribution of latently and/or productively Epstein-Barr virus-infected cells in acute infectious mononucleosis: Implications for the interindividual infection route of Epstein-Barr virus. Blood 85:744–750.

Baer R, Bankier AT, Biggin MD, Deininger PL, Farrell PJ, Gibson TJ, Hatfull G, Hudson GS, Satchwell SC, Seguin C, Tuffnell P, Barrell B (1984): DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature 310:207–211.

Cleary ML, Epstein MA, Finerty S, Dorfman RF, Bornkamm GW, Kirkwood JK, Morgan AJ, Sklar J (1985): Individual tumors of multifocal EBV-induced malignant lymphomas in tamarins arise from different B-cell clones. Science 228:722–724.

Conway M, Morgan AJ, Mackett M (1989): Expression of Epstein-Barr virus membrane antigen gp340/220 in mouse fibroblasts using a bovine papillomavirus vector. Journal of General Virology 70:729–734.

Cox C, Chang S, Karran L, Griffin B, Wedderburn N (1996). Persistent Epstein-Barr virus infection in the common marmoset (*Callithrix jacchus*). Journal of General Virology 77:1173–1180.

Cox C (1997): "Persistent EBV infection in the common marmoset: Effect of immunization with envelope glycoprotein gp340" Ph.D. Thesis. University of London UK.

de Thé G, Geser A, Day NE, Turkei PM, Williams EH, Beri DP, Smith PG, Dean AG, Bornkamm GW, Feorino P, Henle W (1978): Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Ugandan Prospective Study. Nature 274:756–761.

de Thé (1982): Epidemiology of Epstein-Barr virus and associated diseases in man. In Roizman B (ed). "The Herpesviruses." Vol. 1. New York: Plenum Publishing Corp. pp 25–101.

Desgranges C, Lenoir G, de Thé G, Seigneurin JM, Hilgers J, Dubouch P (1976): In vitro transforming activity of EBV 1: Establishment and properties of two EBV strains (M81 and M72) produced by immortalized *Callithrix jacchus* lymphocytes. Biomedicine 25:349–352.

Emini EA, Luka J, Armstrong ME, Banker FS, Provost PJ, Pearson GR (1986): Establishment and characterization of a chronic infectious mononucleosis-like syndrome in common marmosets. Journal of Medical Virology 18:369–379.

Emini EA, Schleif WA, Silberklang M, Lehman D, Ellis RW (1989): Vero cell-expressed Epstein-Barr virus (EBV) gp350/220 protects marmosets from EBV challenge. Journal of Medical Virology 27: 120–123.

Epstein MA (1976): Epstein-Barr virus: Is it time to develop a vaccine program? Journal of the National Cancer Institute 56:697–700.

Epstein MA, Randle BJ, Finerty S, Kirkwood JK (1986): Not all potently neutralizing, vaccine-induced antibodies to EB virus ensure protection of susceptible experimental animals. Clinical and Experimental Immunology 63:485–490.

Farrell PJ, Hollyoake M, Niedobitek G, Agathanggelou A, Morgan A, Wedderburn N (1997): Direct demonstration of persistent Epstein-Barr virus gene expression in peripheral blood of infected common marmosets and analysis of virus infected tissues in vivo. Journal of General Virology 78:1417–1424.

Finerty S, Tarlton J, Mackett M, Conway M, Arrand JR, Watkins PE, Morgan AJ (1992): Protective immunization against Epstein-Barr virus-induced disease in cotton-top tamarins using the virus envelope glycoprotein gp340 produced from a bovine papillomavirus expression vector. Journal of General Virology 73:449–453.

Finerty S, Mackett M, Arrand JR, Watkins PE, Tarlton J, Morgan AJ (1994): Immunization of cottontop tamarins and rabbits with a candidate vaccine against the Epstein-Barr virus based on the major viral envelope glycoprotein gp340 and alum. Vaccine 12: 1180–1184.

Gratama JW, Oosterveer MAP, Zwann FE, Lepoutre J, Klein G, Ernberg I (1988): Eradication of Epstein-Barr virus by allogeneic bone marrow transplantation: Implications for sites of viral latency. Proceedings of the National Academy of Sciences U.S.A. 85:8693–8696.

Gu SY, Huang TM, Ruan L, Miao YH, Lu H, Chu CM, Motz M, Wolf H (1995): First EBV vaccine trial in humans using recombinant vaccinia virus expressing the major membrane antigen. Developments in Biological Standardisation 84:171–177.

Henle G, Henle W (1966): Immunofluorescence in cells derived from Burkitt's Lymphoma. Journal of Bacteriology 91:1248–1256.

Israel ZR, Edmonson PF, Maul DH, O'Neil SP, Mossmann SP, Thiriart C, Fabry L, van Opstal O, Bruck C, Bex F, Burny A, Fultz PN, Mullins JI, Hoover EA (1994): Incomplete protection, but suppression of virus burden, elicited by subunit simian immunodeficiency virus vaccines. Journal of Virology 68:1843–1853.

Johnston DA, Knight AM, Naylor BA, Wedderburn N, Mitchell GH (1990): Monoclonal antibodies from Epstein-Barr virus-transforming lymphocytes of common marmosets (*Callithrix jacchus*) immune to malaria. Journal of Immunological Methods. 127: 187–195.

Khan G, Miyashita EM, Yang B, Babcock GJ, Thorley-Lawson DA (1996): Is EBV persistence in vivo a model for B-cell homeostasis? Immunity 5:173–179.

Labrecque LG, Barnes DM, Fentiman IS, Griffin BE (1995): Epstein-Barr virus in epithelial cell tumors: A breast cancer study. Cancer Research 55:39–45.

Lees JF, Goodeve AC, Arrand JE, Ghosh AK, Jones PH, Arrand JR (1992): Detection of EBV DNA in postnasal space biopsy tissue from asymptomatic EBV-seropositive individuals. Journal of Medical Virology 37:30–38.

Mackett M, Cox C, Pepper S de V, Lees JF, Naylor BA, Wedderburn N, Arrand JR (1996): Immunization of common marmosets with vaccinia virus expressing Epstein-Barr virus (EBV) gp340 and challenge with EBV. Journal of Medical Virology 50:263–271.

Madej M, Conway MJ, Morgan AJ, Sweet J, Wallace LE, Qualtiere LF, Arrand JR, Mackett M (1992): Purification and characterization of Epstein-Barr virus gp340/220 produced by a bovine papillomavirus expression vector system. Vaccine 10:777–782.

Miyashita EM, Yang B, Babcock GJ, Thorley-Lawson DA (1997): Identification of the site of Epstein-Barr virus persistence in vivo as a resting B-cell. Journal of Virology 71:4882–4891.

Morgan AJ, Mackett M, Finerty S, Arrand JR, Scullion FT, Epstein MA (1988): Recombinant vaccinia virus expression Epstein-Barr virus glycoprotein gp340 protects cottontop tamarins against EB virus-induced malignant lymphomas. Journal of Medical Virology 25:189–195.

Morgan AJ, Allison AC, Finerty S, Scullion FT, Byars NE, Epstein MA (1989): Validation of a first-generation Epstein-Barr virus vaccine preparation suitable for human use. Journal of Medical Virology 29:74–78.

Morgan AJ (1992): Epstein-Barr vaccines. Vaccine 10:563–571.

Niedobitek G, Agathanggelou A, Finerty S, Tierney R, Watkins PE, Jones EL, Morgan AJ, Young LS, Rooney N (1994): Latent Epstein-Barr virus infection in cotton-top tamarins. American Journal of Pathology 145:969–978.

Parker BD, Bankier A, Satchwell S, Barrell B, Farrell PJ (1990): Sequence and transcription of Raji Epstein-Barr virus DNA spanning the B95-8 deletion region. Virology 179:339–346.

Pearson G, Henle G, Henle W (1970): Production of antigens associated with Epstein-Barr virus in experimentally infected lymphoblastoid cell lines. Journal of the National Cancer Institute 46: 1243–1250.

Pearson G, Luka J (1986): Characterization of virus determined antigens. In Epstein MA, Achong BG [eds]: "The Epstein-Barr virus recent advances." London: William Heinemann Medical Books, pp 48–73.

Ragot T, Finerty S, Watkins PE, Perricaudet M, Morgan AJ (1993): Replication defective recombinant adenovirus expressing Epstein-Barr virus (EBV) envelope glycoprotein gp340/220 induces protective immunity against EBV-induced lymphomas in the cotton-top tamarin. Journal of General Virology 74:501–507.

Randle BJ, Epstein MA(1984): A highly sensitive enzyme-linked immunosorbent assay to quantitate antibodies to Epstein-Barr virus membrane antigen gp340. Journal of Virological Methods 9:201–208.

Sutjipto S, Pedersen NC, Miller CJ, Gardner MB, Hanson CV, Gettie A, Jennings M, Higgins J, Marx PA (1990): Inactivated simian immunodeficiency virus vaccine failed to protect rhesus macaques from intravenous or genital mucosal infection but delayed disease in intravenously exposed animals. Journal of Virology 64:2290–2297.

Spring SB, Hascall G, Gruber J (1996): Issues related to development of Epstein-Barr virus vaccines. Journal of the National Cancer Institute 88:1436–1441.

Tanner J, Whang Y, Sample J, Sears A, Kieff E (1988): Soluble gp350/220 and deletion mutant glycoproteins block Epstein-Barr virus adsorption to lymphocytes. Journal of Virology 62:4452–4464.

Wedderburn N, Edwards JMB, Desgranges C, Fontaine C, Cohen B, de Thé G (1984): Infectious mononucleosis-like response in common marmosets infected with Epstein-Barr virus. Journal of Infectious Diseases 150:878–882.

Yao QY, Rickinson AB, Epstein MA (1985a): A reexamination of the Epstein-Barr virus carrier state in healthy seropositive individuals. International Journal of Cancer 35:35–42.

Yao QY, Rickinson AB, Gaston JSH, Epstein MA (1985b): In vitro analysis of the Epstein-Barr virus: Host balance in long-term renal allograft recipients. International Journal of Cancer 35:43–49.

Yao QY, Ogan P, Rowe M, Wood M, Rickinson AB (1989): The Epstein-Barr virus: Host balance in acute infectious mononucleosis patients receiving acyclovir antiviral therapy. International Journal of Cancer 43:61–66.